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Cloning and Sequence Analysis of a cDNA Encoding *Pso o* II, a Mite Group II Allergen of the Sheep Scab Mite (Acari: Psoroptidae)

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ABSTRACT *Psoroptes ovis* (Hering), the sheep scab mite, is responsible for psoroptic scabies of cattle and sheep. Reverse translation of 30 N-terminal amino acids of the major *P. ovis* allergen, previously chosen as a candidate immunogen and identified as a 16 kDa protein yielded a degenerate sequence used to design oligodeoxynucleotide polymerase chain reaction (PCR) primers. Use of the PCR primers with a *P. ovis* cDNA library succeeded in amplification of a 90 bp cDNA gene fragment that was cloned, sequenced, and used to select unique sequencing/PCR primers. Primer walking generated overlapping subclones which yielded the 588 nucleotide consensus sequence of the cDNA encoding the 143 amino acid *P. ovis* allergen precursor. Nucleotide and translated sequences of the cDNA were compared with sequences in GenBank and found to be homologous to mite group II allergens *Lep d* II (formerly *Lep d* I) of *Lepidoglyphus destructor* Schrank, *Der f* II of *Dermatophagoides farinae* Hughes, *Der p* II of *Dermatophagoides pteronyssinus* (Trouessart), *Tyr p* II of *Tyrophagus putrescentiae* (Schrank), *Eur m* II of *Euroglyphus maynei* (Cooreman) and *Gly d* II of *Glycophagus domesticus* (De Geer). The mature *P. ovis* allergen is composed of 126 amino acids with a calculated molecular mass of 13,468 Da, three disulfide bonds, and pI of 6.06 with one potential o-glycosylation site at Thr116. We designate the *P. ovis* 16 kDa protein as *Pso o* II in conformity with nomenclature for mite group II allergens.

RESUMEN *Psoroptes ovis* (Hering), el ácaro de la sarna de los ovinos, es responsable de la sarna psoróptica del ganado y las ovejas. Traducción reversa de 30 amino ácidos de la región N-terminal del alérgeno mayor de *P. ovis*, fue previamente escogido como un candidato a immunógeno e identificado como una proteína de 16 kDa, de la cual se diseñaron oligonucleótidos iniciadores degenerados para PCR. El uso de los iniciadores con una geneteca de cDNA de *P. ovis*, fueron exitosos para amplificar un fragmentode cDNA de 90 pares de bases, los cuales fueron clonados, secuenciados y usados para seleccionar iniciadores específicos para PCR. Iniciadores detraslape de secuencias generaron subclonas, las cuales resultaron en una secuencia consenso de cDNA de 588 nucleótidos, que codifica el alergeno precursor de 143 amino ácidos de *P. ovis*. Las secuencias traducidas de nucleótidos de cDNA fueron comparadas con secuencias en el GenBank y se encontraron homólogas al grupo II de alergenos de ácaros *Lep d* II (antes *Lep d* I) de *Lepidoglyphus destructor* (Schrank), *Der f* II de *Dermatophagoides farinae* (Hughes), *Der p* II de *Dermatophagoides pteronyssinus* (Trouessart), *Tyr p* II de *Tyrophagus putrescentiae* (Schrank), *Eur m* II de *Euroglyphus maynei* (Cooreman) y *Gly d* II de *Glycophagus domesticus* (De Geer). El alergeno maduro de *P. ovis* esta compuesto de 126 amino ácidos con una masamolecular calculada de 13,468 Da, three puentes disulfuro y un pI de 6.06, con un sitio potencial de o-glicosilación en Thr116. Nosotros designamos la proteínade 16 kDa de *P. ovis* como *Pso o* II para concordar con la nomenclatura para el grupo II de alergenos de ácaros.

KEY WORDS sheep scab mite, psoroptic scabies, mite group II allergens

Psoroptes ovis (HERING), commonly known as the sheep scab mite, is the causative agent of psoroptic scabies (psoroptic mange) of cattle and sheep, a world-wide problem and a quarantinable disease in the United States with estimated 1976 losses in the

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In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Institutional Animal Use and Care Committee (IAUCC) of the Knipling-Bushland U. S. Livestock Insects Research Laboratory, Kerrville, TX.

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United States of over $250 million (Hall 1985, Wright 1985). Psoroptic scabies is an allergic dermatitis (Stromberg and Guillot 1989). Infested animals may exhibit substantial scab development and loss of hair and skin, potentially leading to death of the animal either directly or as a result of general decline and increasing susceptibility to secondary infections. Development and progression of psoroptic scabies in susceptible animals is dependent on production of antibodies, development of an inflammation response, and loss of serous exudate from the skin of the infested animal. Mite populations increase dramatically following development of the serous exudate. Precise conditions necessary for this rapid increase in mite population are unknown, however, immunosuppression of infested animals with dexamethasone prevented animals from developing a lesion scab in response to the mites, and mite populations did not exhibit the normal proliferation (Pruett et al. 1989). Initial vaccination trials using *P. ovis* immunogens resulted in altered immune responses upon infestation and altered clinical progression (Pruett et al. 1998). These results suggest that a particular immune response in naïve cattle may facilitate scabies development, and that alteration of the normal response by prior vaccination may affect mite population growth and lesion development.

Development of test vaccines requires a source of purified mite antigens, suggesting use of recombinant DNA technology for large-scale production. A major *P. ovis* allergen, identified as a 16 kDa protein, was chosen as a candidate immunogen, purified, and sequence of the first 30 N-terminal amino acids was obtained (Pruett 1999). This report describes the cloning, sequencing and analysis of the cDNA encoding the 16 kDa *P. ovis* allergen.

Materials and Methods

Mites. *Psoroptes ovis* mites were obtained from a laboratory colony maintained on stanchioned cattle as described previously (Pruett et al. 1998). Mites were collected, rinsed with 10 mM Tris-HCl containing 25 mM EDTA, pH 7.5, and stored in liquid nitrogen until use.

Total RNA. Total RNA was isolated from frozen mites by grinding in a Tenbroeck tissue grinder with hot (80°C) acid phenol as described previously (Temeyer and Pruett 1990) or in RNA Isolator (Genosys Biotechnologies, The Woodlands, TX) at 80°C or 23°C according to the manufacturer's instructions.

Messenger RNA. Messenger RNA was separated from total RNA using either the PolyATract mRNA isolation kit (Promega, Madison, WI), or oligo(dT)-cellulose columns (type 3, Collaborative Biomedical Products, Becton Dickinson Labware, Bedford, MA), according to the manufacturers' instructions. Messenger RNA integrity was tested by in vitro translation with or without addition of canine as pancreatic microsomes (Promega) to assess signal peptide cleavage and glycosylation as previously described (Temeyer and Pruett 1990).

cDNA. Double stranded cDNA (ds-cDNA) was synthesized from messenger RNA template using the SuperScript Choice kit (Life Technologies, Gaithersburg, MD) following instructions essentially as specified by the manufacturer. *Not*I/*Eco*RI adapters were ligated onto the ends of the ds-cDNA and unincorporated adapters were removed by sephadex column chromatography. cDNA was then ligated to λ-ZipLox *Eco*RI arms (Life Technologies) and packaged into λ phage using the Packagene Lambda DNA Packaging System (Promega) or MaxPlax packaging extract (Epicentre Technologies, Madison, WI). The resulting cDNA libraries were titered on LB-agar plates containing ampicillin at 100 μg/ml using *E. coli* Y1090(ZL) as the recipient host strain.

Oligodeoxynucleotide Synthesis. Oligodeoxynucleotide sequences were selected for use in PCR or sequencing with the aid of Oligo Primer Analysis Software (version 5.0 for Macintosh, National Biosciences, Plymouth, MN). Oligodeoxynucleotides were synthesized by Genosys Technologies (The Woodlands TX).

PCR. PCR was performed using ampliTaq Gold (Perkin-Elmer, Foster City, CA) or recombinant *Taq* polymerase (Life Technologies) according to the manufacturer's instructions. Cycle parameters routinely involved hot start with polymerase addition or activation during 1 min at 94°C, anneal 1 min at 55–60°C and 2 min extension at 72°C for 30–40 cycles. PCR amplification products were cloned by ligation into plasmid pNoTA and transformation into *E. coli* JM109 with selection on LB-agar plates containing 100 μg ampicillin/ml according to instructions supplied with the Prime PCR Cloner (5 Prime → three Prime, Boulder, CO) cloning system. DNA was electrophoretically analyzed on agarose gels using standard techniques.

Dideoxynucleotide Sequencing. Nucleotide sequences were obtained using oligodeoxynucleotide primers, alpha-[P-33]-dATP (DuPont NEN Life Science Products, Boston, MA) and the Sequenase version 2.0 DNA sequencing kit (USB, Cleveland OH) according to the manufacturer's instructions. DNA sequencing gels contained 6% Long Ranger gel solution (FMC BioProducts, Rockland, ME) and 7 M urea (Sigma, St. Louis, MO) in glycerol tolerant gel buffer (Amersham Pharmacia Biotech, Piscataway, NJ). DNA sequence was obtained from autoradiograms of sequencing gels dried under vacuum on blotting paper at 80°C and exposed to Kodak BioMax MR Scientific Imaging Film (Sigma) at −70°C.

Sequence Analysis. Sequence data were analyzed using MacVector sequence analysis software version 6.0 for Macintosh (Oxford Molecular Group, Oxford, UK). Consensus sequences were derived by agreement between sequences from multiple overlapping subclones. The consensus cDNA sequence was analyzed to identify open reading frames for the presumptive translation product, which was further analyzed using the MacVector protein toolbox. The 143 amino acid sequence deduced from the consensus cDNA sequence (Fig. 4) was submitted to a similarity search using BLASTP 2.1.1 [8 August 2000] (Altschul et al. 1997) and was compared with 576,719 protein

sequences compiled from GenBank, CDS translations, PDB, SwisProt, SPupdate and PIR. The complete cDNA sequence (Fig. 4, GenBank accession BankIt283560 AF187083) was also submitted to a similarity search using BLASTN 1.4.11 [24 November 1997] (Altschul et al. 1990) and was compared with 343,316 nonredundant nucleotide sequences consisting of 706, 578,648 total nucleotides.

Protein Processing and Modification. Modification of the *P. ovis* allergen by glycosylation, acylation, myristoylation, or other known recognition motifs was investigated by searching the amino acid sequence using software available through the BCM Search Launcher (Smith et al. 1996; available at http://www.hgsc.bcm.tmc.edu/SearchLauncher/), Signal-P (Nielsen et al. 1997; available at http://www.cbs.dtu.dk/services/SignalP/), NetOGlyc 2.0 (Hansen et al. 1995, 1997, 1998; available through the BCM Search Launcher), ProDom (available through the BCM Search Launcher), and other sequence analysis tools.

Multiple Alignment and Relatedness of Mite Group Two Allergens. Primary structure relatedness of mite group II allergens was investigated through use of Clustal W 1.81 (Thompson et al. 1994; available through the European Bioinfomatics Institute at http://www2.ebi.ac.uk/clustalw) and other software for multiple alignment, protein analysis and family identification such as BLOCKS (Henikoff and Henikoff 1994, Henikoff et al. 1995) (BLKPROB version 8:26:99.1, available through the BCM Search Launcher).

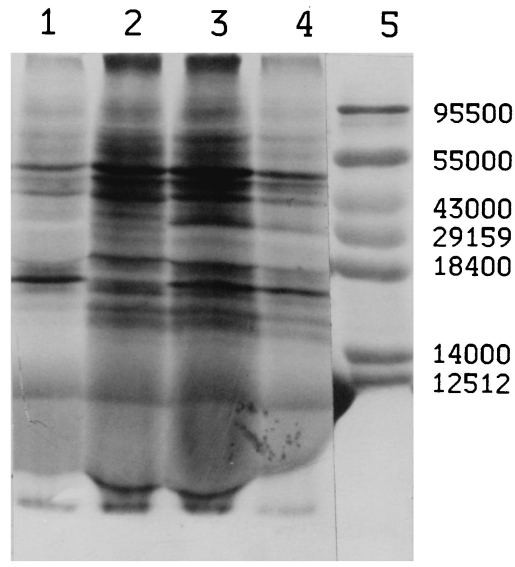

Fig. 1. In vitro translations of *Psoroptes ovis* mRNA were separated electrophoretically on a 12% polyacrylamide gel as described (Temeyer and Pruett 1990). Lanes 1–4: autoradiogram. Lane 5: Coomassie blue-stained gel. Messenger RNAs were added to in vitro translations as follows. Lane 1: 2 μl oligo(dT)cellulose mRNA. Lane 2: 2 μl PolyATract mRNA with canine pancreatic microsomes. Lane 3: 2 μl PolyATract mRNA. Lane 4: 1 μl PolyATract mRNA. Lane 5: protein standards on stained gel (molecular mass of standards is indicated in Daltons).

Results

Alternative methods of isolating RNA from mites were evaluated. Total RNA yield from mites ground in RNA Isolator was approximately two times higher (0.2% [wt:wt] with respect to mites) than obtained using hot acid phenol. The PolyATract mRNA Isolation System yielded ≈3.5 times more poly(A+)-RNA (mRNA) compared with an oligo(dT)-cellulose column and gave optimal yields of mRNA calculated at 0.288% of the total RNA. Messenger RNA isolated using the PolyATract mRNA Isolation System exhibited a broad size range on agarose gel electrophoresis and exhibited greater in vitro translation activity than mRNA isolated from a split sample of total mite RNA using oligo(dT)-cellulose (Fig. 1, lanes 1 and 3). Addition of canine pancreatic microsomes altered the profile of in vitro translated *P. ovis* mRNA-directed protein synthesis (Fig. 1, lane 2), suggesting that several of the major *P. ovis*-specific translation products were cotranslationally or posttranslationally modified. Approximately 1.6 μg *P. ovis* messenger RNA was used to direct cDNA synthesis using an oligo(dT)12–18 primer for first strand synthesis. First strand yield was calculated to be 940 ng.

A major *P. ovis* allergen, previously chosen as a candidate immunogen and identified as a 16 kDa protein, was purified and the sequence of the first 30 N-terminal amino acids was obtained (Pruett 1999). Reverse translation of the N-terminal amino acid sequence (Fig. 2) provided an ambiguous nucleotide sequence, which was used to design degenerate oligodeoxynucleotide primers (Table 1). An additional primer (T16K15–15, Table 1) was synthesized with incomplete homology to the adapter sequence (5'-GTCGACGCGGCCGCG-3', Life Technologies) that had been ligated to the 3'-ends of the ds-cDNA during cDNA library construction. PCR reactions using primer T16K15–15 paired with other primers from Table 1 gave discrete amplification products with size differences corresponding to separation of the individual primers in the 90 bp sequence. This result established that the primers were specific and provided an estimate of the size of the target cDNA. Use of primer pair F16K20–20 and R16K71–21 in PCR reactions succeeded in amplification of a unique 90 bp cDNA gene fragment, which was cloned and sequenced using standard techniques. The unambiguous nucleotide sequence obtained was in agreement with that predicted by reverse translation and was used to select oligonucleotide primers that were used in PCR reactions with primer T16K15–15 to amplify a series of overlapping cDNA fragments anchored at either the 5'- or 3'-end of the cDNA. These cDNA fragments were cloned and sequenced using additional primers (Table 2) based on newly revealed sequence data until both strands of the entire cDNA gene had been sequenced to yield the complete 588 nucleotide consensus sequence of the *P. ovis* 16 kDa allergen cDNA (GenBank accession BankIt283560 AF187083) encoding 143 amino acids (Fig. 3).

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Sequence Range: 1 to 90

          10          20          30          40
           *           *           *           *
GGN AAR GTN AAR TTY CAR GAY TGY GGN AAR GGN GAR GTN GAR WSN
CCN TTY CAN TTY AAR GTY CTR ACR CCN TTY CCN CTY CAN CTY WSN
Gly Lys Val Lys Phe Gln Asp Cys Gly Lys Gly Glu Val Glu Ser>
_Reverse translation of Po16k N-terminal amino acids 1-30_>

    50          60          70          80          90
     *           *           *           *           *
YTN GAR GTN GAR GGN TGY WSN GGN GAY TAY TGY GTN ATH CAY AAR
RAN CTY CAN CTY CCN ACR WSN CCN CTR ATR ACR CAN TAD GTR TTY
Leu Glu Val Glu Gly Cys Ser Gly Asp Tyr Cys Val Ile His Lys>
_Reverse translation of Po16k N-terminal amino acids 1-30_>
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Fig. 2. Reverse translation of N-terminal amino acids 1–30 from mature *P. ovis* 16 kDa protein.

Nucleotide sequence of the cDNA was compared and aligned with sequences in GenBank. Significant homology was found (Table 3) to *L. destructor* mRNA for *Lep d* II (prev. *Lep d* I, Olsson et al. 1998) allergen, *D. farinae* mRNA for mite allergen *Der f* II and *T. putrescentiae* mRNA for group II allergen. Amino acid sequence homology was apparent (Table 3) to group II mite allergens *Lep d* I (now *Lep d* II, Olsson et al. 1998) of *Lepidoglyphus destructor*, *Der f* II of *Dermatophagoides farinae*, *Der p* II of *Dermatophagoides pteronyssinus*, the group II allergen of *Tyrophagus putrescentiae*, *Eur m* II of *Euroglyphus maynei*, and *Gly d* II of *Glycophagus domesticus* (De Geer). Multiple sequence alignment of precursors to the mite group II allergens is shown in Fig. 4. Numbering corresponds to the mature form of *Pso o* II. The homology line indicates the degree of amino acid homology among all aligned sequence members: asterisk = identical or conserved residues, colon = conserved substitutions, and a period = semiconserved substitutions. Results of the multiple sequence alignment were used to construct an unrooted tree (cladogram) using PHYLIP (the *PHYL*ogeny *I*nference *P*ackage; Felsenstein 1989; available at http://evolution.genetics.washington.edu/phylip.html) showing relatedness of amino acid sequences of the mite group II allergen family (Fig. 5). As can be seen, *Pso o* II clearly falls within the mite group II allergens with amino acid sequence most homologous to *Lep d* II, *Gly d* II and *Tyr p* II.

Discussion

Use of commercially available kits provided convenient, reliable methods for successful cDNA library construction. Although the supplier recommends only 10% tissue compared with the volume of RNA Isolator used, we found no reduction in total RNA yield (per gram of mites) or quality (assessed by in vitro translation) at up to 20% mites (wt:vol) with homogenization in hot (80°C) or room temperature reagent. Use of the PolyATract mRNA Isolation System reduced both RNA handling and isolation time, both of which improved mRNA quality by reducing the opportunity for RNase introduction and RNA damage compared with use of oligo(dT) columns. We used the SuperScript Choice System for cDNA synthesis primarily because use of the λ-ZipLox vector offered a simple method for expression of cloned genes for screening purposes, and simple conversion to a plasmid vector for large yield of cloned DNA for subsequent analysis, modification, and subcloning. Random selection of recombinant phage derivatives all appeared to contain cDNA inserts as evidenced by excision with endonuclease *Eco*RI.

The complete sequence of the cDNA encoding the *P. ovis* 16 kDa protein contained an open reading frame beginning with tandem methionine initiation codons and continuing with a total of 17 amino-terminal amino acids not present in the mature protein

Table 1. Initial oligonucleotide primers for PCR and sequencing

Name	Sequence (5′ → 3′)	Position[a]	Length	T_m
R16K19-23	TCNACYTCNCCYTTNCCRCARTC	41 → 19	23	59.4°
R16K52-23	TARTCNCCNSWRCANCCYTCNAC	74 → 52	23	57.1°
R16K71-21	YTTRTGDATNACRCARTARTC	99 → 70	21	47.9°
F16K20-20	GGNAARGTNAARTTYCARGA	1 → 20	20	51.5°
F16K38-23	CARGAYTGYGGNAARGGNGARGT	16 → 38	23	63.2°
F16K80-26	GARGGNTGYWSNGGNGAYTAYTGYGT	55 → 80	26	65.3°
F16K89-23	GGNGAYTAYTGYGTNATHCAYAA	67 → 89	23	54.8°
T16K15-15	CCTCGCGGCCTCGTC	3′-adapter	15	67.4°

T_m = Melting temperature of hybridized oligo. A = Adenine; C = Cytosine; G = Guanine; T = Thymine; D = A, G, or T; H = A, C, or T; N = A, C, G, or T; R = A or G; S = C or G; W = A or T; Y = C or T (Nomenclature Committee of the International Union of Biochemistry).

[a] Relative to position 1 (Fig. 3) of the + strand specifying N-terminal amino acids 1-30.

Table 2. Oligonucleotide primers for PCR and sequencing

Name	Sequence (5′ → 3′)	Position[a]	Length	T_m
F16K1-24	GGGAAGGTCAAGTTTCAAGACTGT	1 → 24	24	65.4°
F16K21-30	CTGTGGAAAAGGGGAAGTTGAATCTCTTGA	21 → 50	30	73.1°
157U23	ATTGTTGCCGATATCAACGGTGT	157 → 179	23	68°
177U27	TGTACAAATTGAAGTTCCTGGCGTTGA	177 → 203	27	71.3°
284U22	CAATCTTGCCAACTACCAAAGC	284 → 305	22	64.5°
R16K34-30	ACAGCCTTCGACTTCAAGAGATTCAACTTC	63 → 34	30	71.3°
R16K60-22	GACGCAGTAGTCACCTGAACAG	81 → 60	22	63.8°
127L28	CGAGTTTCAAATTGGCTGAATCTTGGTT	154 → 127	28	71.5°
184L27	ATCATGATCAACGCCAGGAACTTCAAT	206 → 184	27	71.5°
286L24	TTTAGCTTTGGTAGTTGGCAAGAT	309 → 286	24	63.6°

T_m = Melting temperature of hybridized oligo; A = Adenine; C = Cytosine; G = Guanine; T = Thymine.
[a] Relative to Position 1 (Fig. 3) of the + strand specifying N-terminal amino acids 1-30.

N-terminal sequence reported by Pruett (1999). We investigated the possibility that the 17 N-terminal amino acids not present in the mature protein function may represent a secretion signal peptide.

Multiple alignment of the mite group II allergen precursors (Fig. 4) demonstrated significant homology with identified signal peptides for *Der f* II (17 amino acid signal peptide) and *Lep d* II (16 amino acid signal peptide). Alignment of the signal peptide of Po16k with the mite allergen Der F7 precursor (GenBank accession Q26456, data not shown) revealed 8/17 = 47% identities and 15/17 = 88% positive sim-

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        10         20         30         40         50         60         70
CCTCGCGGCCTCGTCGACCCCAATTAAAACTAAAAAATAATTTTAAAAAAATCAAAATGATGAAAACTTT
                                                    M  M  K  T  L
                                                                       5

        80         90        100        110        120        130        140
GGTAGTTCTCGCCATCACTTTGGCTGTTGTATCAGCTGGSAARGTCAARTTYCAAGACTGTGGAAAAGGR
  V  V  L  A  I  T  L  A  V  V  S  A  G  K  V  K  F  Q  D  C  G  K  G
              10                 15                 20                 25

       150        160        170        180        190        200        210
GAAGTTGAATCTCTTGAAGTTGAAGGCTGTTCAGGTGATTACTGCGTCATTCACAAAGGTAAAAAACTTG
 E  V  E  S  L  E  V  E  G  C  S  G  D  Y  C  V  I  H  K  G  K  K  L
    30                 35                 40                 45                 50

       220        230        240        250        260        270        280
ATTTAGCCATCAGTGTAACATCGAACCAAGATTCAGCCAATTTGAAACTCGATATTGTTGCCGATATCAA
 D  L  A  I  S  V  T  S  N  Q  D  S  A  N  L  K  L  D  I  V  A  D  I  N
          55                 60                 65                 70                 75

       290        300        310        320        330        340        350
CGGTGTACAAATTGAAGTTCCTGGCGTTGATCATGATGGTTGCCATTACGTCAAATGTCCAATCAAGAAA
  G  V  Q  I  E  V  P  G  V  D  H  D  G  C  H  Y  V  K  C  P  I  K  K
              80                 85                 90                 95

       360        370        380        390        400        410        420
GGCCAACACTTTGACGTCAAATACACATACAGCATTCCAGCAATCTTGCCAACTACCAAAGCTAAAATCA
 G  Q  H  F  D  V  K  Y  T  Y  S  I  P  A  I  L  P  T  T  K  A  K  I
    100                105                110                115                120

       430        440        450        460        470        480        490
TTGCTAAAATTATTGGTGATAAAGGTCTTGGTGGTTGTATCGTAATCAATGGTGAAATTCAAGACTAAAT
I  A  K  I  I  G  D  K  G  L  G  G  C  I  V  I  N  G  E  I  Q  D  ***
          125                130                135                140

       500        510        520        530        540        550        560
CAATAAAAACCTAAAAATATTTTGATGAAATTAGATTTGTTATTTTTATTTCTCATTTTATTCAAAATTA

       570        580
AAAAGTATTCAGTCGACGAGGCCGCGAG
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Fig. 3. Nucleotide sequence of the cDNA gene encoding the *P. ovis* 16 kDa allergen, *Pso o* II. The 588 nucleotide sequence and numbering of the cDNA is listed above the encoded amino acid sequence and numbering. The underlined 143 amino acid sequence corresponds to the mature Po16k allergen, *Pso o* II. Disulfide bond positions are specified in Fig. 4. The 17 amino acid leader sequence (italics) is consistent with a signal sequence expected for a secreted protein.

Table 3. Po16k amino acid sequence homology to mite group II allergens

Mite allergen	Database accession code[a]	References	Nucleotide sequence identity	E value	AA sequence identity	AA sequence similarity	E value
Der f II	AAB30829 148475 D10447	Yuuki et al. 1991	(133/227) 58%	7.8e-14	(43/100) 43%	(70/100) 70%	1e-22
Der p II	P49278 AAF86462	Chua et al. 1990, Heymann et al. 1989, Mueller et al. 1997, 1998	—	—	(41/98) 41%	(68/98) 69%	2e-21
Eur m II	AF047613 AAC82349	Smith et al. 1999	—	—	(44/101) 44%	(70/101) 69%	1e-21
Gly d II	CAB76459	Gafvelin et al. 2001	—	—	(53/98) 54%	(71/98) 72%	2e-28
Lep d II	X83875 P80384	Schmidt et al. 1995, Varela et al. 1994, Muthiah et al. 1991, van Hage-Hamsten et al. 1992	(204/358) 56%	8.4e-25	(52/127) 41%	(80/127) 63%	5e-28
Tyr p II	Y12690 O02380	Eriksson et al. 1998	(167/319) 52%	2.6e-10	(52/127) 40%	(80/127) 62%	1e-25

AA = amino acid.
[a] GenBank, SwissProt, EMBL or other databases.

ilarities. Of these, the first three amino acids and the three amino acids preceding the presumptive cleavage site were identical. Analysis of amino acid sequences using Signal-P (Nielsen et al. 1997) to identify presumptive eukaryotic signal peptides and cleavage points identified the first 17 amino acids of Po16k as a signal sequence with cleavage between amino acids 17–18 in agreement with our characterization. Simi-

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Pso o II mature amino acid sequence range: 1 to 126

                              ‡         10        20         30        40
Der p II    MMYKILCLSLLVAAVA--RDQVDVKDCANHEIKKVLVPGCHGSEPCIIHRGKPFQLEAVF
Eur m II    -MYKILCLSLLVAAVAA--DQVDVKDCANHEIKKVMVPGCKGSEPCVIHRGTAFQLEAVF
Der f II    MISKILCLSLLVAAVVA/-DQVDVKDCANNEIKKVMVDGCHGSDPCIIHRGKPFTLEALF
Lep d II    MMKFIALFAL-VAVASA/-GKMTFKDCGHGEVTELDITGCSG-DTCVIHRGEKMTLEAKF
Gly d II    -------------------GKMKFKDCGKGEVTELDITDCSG-DFCVIHRGKPLTLEAKF
Pso o II    MMKTLVVLAITLAVVSA/-GKVKFQDCGKGEVESLEVEGCSG-DYCVIHKGKKLDLAISV
Tyr p II    -MKFLILFAL-VAVAAA--GQVKFTDCGKKEIASVAVDGCEG-DLCVIHKSKPVHVIAEF
homology     :  :  ::: :*..    .:: . **.: *: .: : .* * : *:**:.  . :   .
S-S bond                                1            2     2

                     50        60        70        80        90        100
Der p II    EANQNTKTAKIEIKASIDGLEVDVPGIDPNACHYMKCPLVKGQQYDIKYTWNVPKIAPKS
Eur m II    DANQNSNAAKIEIKATIDGVEIDVPGIDNNLCHFMKCPLVKGQEYDIKYTWNVPRIAPKS
Der f II    DANQNTKTAKIEIKASLDGLEIDVPGIDTNACHFMKCPLVKGQQYDIKYTWNVPKIAPKS
Lep d II    AANQDTAKVTIKVLAKVAGTTIQVPGLETDGCKFIKCPVKKGEALDFIYSGTIPAITPKV
Gly d II    AANQDTTKATIKVLAKVAGTPIQVPGLETDGCKFVKCPIKKGDPIDFKYTTTVPAILPKV
Pso o II    TSNQDSANLKLDIVADINGVQIEVPGVDHDGCHYVKCPIKKGQHFDVKYTYSIPAILPTT
Tyr p II    TANQDTCKIEVKVTGQLNGLEVPIPGIETDGCKVLKCPLKKGTKYTMNYSVNVPSVVPNI
homology     :**::    :.: . : *  : :**:: : *: :***: **    . *: .:* : *.
S-S bond                                      3    3

                    110       120
Der p II    ENVVVTVKVMGDDGVLACAIATHAKIRD
Eur m II    ENVVVTVKLLGDNGVLACAIATHAKIRD
Der f II    ENVVVTVKLIGDNGVLACAIATHGKIRD
Lep d II    K-ADVTAELIGDHGVMACG-TVHGQVE-
Gly d II    K-AEVTAELVGDHGVLACG-RFGRQVE-
Pso o II    K-AKIIAKIIGDKGLGGCI-VINGEIQD
Tyr p II    K-TVVKLLATGEHGVLACG-AVNTDVKP
homology    : . :     *:.*: .*      .:.
S-S bond                  1
```

Fig. 4. Multiple sequence alignment for homologous mite group II allergen precursors.

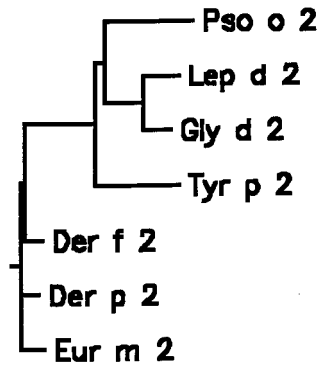

Fig. 5. Cladogram based on multiple amino acid sequence alignment by Clustal W 1.81.

larly, Signal-P analysis predicted that all of the mite group II allergen precursors listed in Fig. 4 have signal sequences with predicted cleavage points (indicated by ‡) shown in the numbering line for Po16k above the aligned sequences. Cleavage sites of known signal sequences are indicated by a / within the aligned amino acid sequences.

The protein analysis toolbox of MacVector (version 6.0) calculated the molecular mass of the 126 amino acid mature protein at 13,468 d with an estimated pI of 6.06. This result appears to disagree with the initial characterization of the *P. ovis* allergen as a 16 kDa protein by SDS-PAGE (Pruett 1999), however a number of reports indicate that the apparent molecular mass of small mite antigens is highly dependent upon the technique used (Lind 1985, Heymann et al. 1989, Varela et al. 1994). In addition, NetOGlyc 2.0 (Hansen et al. 1995, 1997, 1998) analysis suggested one possible o-glycosylation site at Thr116. There were no N-glycosylation sites containing the recognition sequences Asn-Xaa-Thr/Ser or Asn-Xaa-Cys (Marshall 1972, Pless and Lennarz 1977, Bause 1983, Gavel and von Heijne 1990, Miletich and Broze 1990), nor were there recognition sequences for N-myristoylation (Towler et al. 1988, Grand 1989).

Previous studies have reported antigenic cross-reactivity between *Psoroptes* and *Dermatophagoides* mites (Fisher 1972, Stewart and Fisher 1986). The multiple alignment demonstrates significant homology throughout the amino acid sequence, and also demonstrates complete conservation of the aligned Cys residues, suggesting conservation of the disulfide linkages for all of the mite group II allergens similar to that reported for *Der f* II (Nishiyama et al. 1993) between mature *Der f* II Cys residue pairs [8, 119], [21, 27], and [73, 78], corresponding to mature *P. ovis* allergen Cys residues [8, 117], [21, 26], and [73, 78]. Similar disulfide bond linkages have also been reported for *Der p* II (Mueller et al. 1997, 1998), *Tyr p* II (GenBank Accession O02380) and *Lep d* II (GenBank Accession P80384).

The similarity search program, BLOCKS (Henikoff and Henikoff 1994, Henikoff et al. 1995), was used to identify protein families as an aid in characterization of unknown amino acid sequences. Use of the BLOCKS program identified amino acid sequence blocks characteristic of the mite group II allergens (including Po16k) that do not belong to any other previously identified protein family. Similarly, Protomap (release 3.0, May 2000, at http://protomap.stanford.edu/) also places *Pso o* II within the mite group II allergens in cluster 2878.

Mite group II allergens are small (≈14 kDa) neutral to basic proteins with three internal disulfide bonds that are essential to their immunogenicity (Robinson et al. 1997). The designation of *Lep d* I has been changed to *Lep d* II in recognition of its homology with mite group II allergens (Olsson et al. 1998). We propose that the major *Psoroptes ovis* allergen previously identified as Po16k (Pruett 1999) be designated as *Pso o* II in conformance to the nomenclature used for homologous mite group II allergens.

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